EXHIBIT Y

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Degradation of polypropylene *in vivo*: A microscopic analysis of meshes explanted from patients

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Abstract: Polypropylene meshes, originally introduced for hernia repair, are presently utilized in several anatomical sites. Several million are implanted annually worldwide. Depending on the device, up to 10% will be excised to treat complications. The excised meshes can provide material to study the complications, however, they have remained underutilized over the last decades and the mechanisms of complications continue to be incompletely understood. The fundamental question as to whether polypropylene degrades *in vivo* is still debated. We have examined 164 excised meshes using conventional microscopy to search for features of polypropylene degradation. Four specimens were also examined by transmission electron microscopy. The degraded material, detected by its ability to absorb dyes in the degradation nanopores, formed a continuous layer at the surface of the mesh fibers. It retained birefringence, inclusions of non-degraded polypropylene, and showed ability to meld with the non-degraded fiber core when heated by the surgical cautery. Several features indicated that the degradation layer formed *in vivo*: inflammatory cells trapped within fissures, melting caused by cautery of excision surgery, and gradual but progressive growth of the degradation layer while in the body. Cracking of the degraded material indicated a contribution to clinically important mesh stiffening and deformation. Chemical products of degradation need to be analyzed and studied for their role in the mesh-body interactions. The described methods can also be used to study degradation of other materials. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2015.

Key Words: polypropylene, mesh, degradation, pathology, microscopy, hernia, vaginal

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INTRODUCTION

Polypropylene meshes were introduced in the late 50s for hernia repair.[1] Over the next decades, their use spread to other anatomical sites. Presently, they are employed in millions of surgeries worldwide and this expansive use has been catalyzed by the rapid proliferation of new and minimally invasive surgical techniques.[2,3] Depending on the anatomical site, in published reports to date, 2–10% of implanted meshes are explanted or partially excised for complications such as pain, infection, erosion through the vaginal mucosa and other adjacent structures, urinary symptoms or recurrence of a herniation.[4–9] These excision specimens generate a large, but underutilized body of study material. Surprisingly, there are very few studies reporting findings of polypropylene explants from patients. A small number of animal studies, which are more expensive to conduct, have been published; however these had the obvious limitations associated with animal experiments. This has created a paradoxical situation in which despite the long history of use and the large volume of explanted polypropylene devices, the causes and mechanisms of complications associated with the mesh remain incompletely understood. For example, the fundamental question as to whether polypropylene degrades *in vivo* is still unresolved 50 years after its introduction as an implantable material.[10,11]

Degradation of polymers and polypropylene more specifically has been studied outside the medical field. The typical features of changing appearance of degrading polymers are cracks and irregularity of the surface.[12–14] Polymer breakdown can be caused by physical factors, such as high temperature and ultraviolet light, as well as chemical factors, such as oxidation. Biodegradation, a form of chemical degradation, has been studied outside of medical settings as bacterial degradation of polypropylene discarded in the environment. The study revealed polypropylene surface changes similar to those induced by the physical factors.[15] In animals, the initial experiments which used mechanical and spectral methods of testing, showed that polypropylene fibers underwent oxidative degradation when implanted in mammals. The detected changes were similar to autoxidation of the polymer at elevated temperatures.[16] In that study chemical induction of the surface was observed at 108 days for unstabilized polypropylene, while induction was delayed by the addition of antioxidants during the

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TABLE I. Sample and Patient Data

Transvaginal			Patient Age Years, Median (Range)	Mesh *In Vivo* Months, Median (Range)	Symptoms (%)		
					Pain, Dyspareunia	Erosion	Urinary Symptoms
Slings, *n*			52 (25–71)	48 (10–108)	57	38	33
AMS[a] Sparc/Monarc	21	69					
BSC[b] Obtryx/Adv.	16						
Ethicon TVT/TVT-O	28						
Bard Align	4						
Pelvic organ prolapse, *n*							
AMS Apogee/Perig.	16	42					
BSC pinnacle/uphold	8						
Ethicon prolift	4						
Bard avaulta	9						
Undetermined	5						

HERNIA					Symptoms (%)			
					Pain	Recurrence	Infection	Migration into organs
Inguinal, *n*			47 (24–82)	36 (3–169)	48	52	10	6
Ethicon Prolene	3	37						
Bard Marlex	5							
Undetermined	29							
Ventral, *n*								
Undetermined		16						

[a] American Medical Systems.
[b] Boston Scientific.

manufacturing process. In the body, oxidative degradation facilitated by macrophages surrounding the mesh fibers was thought to be the major contributor.[17–20] When scanning electron microscopy was introduced into the field, the studies focused on surface changes of explanted polypropylene.[21–29] These reports showed cracking and scaling of the surface, both of which became the subject of interpretation and speculation. Alternative explanations were either cracking of a biological material formed by deposited proteins, or polypropylene degradation induced by chemical and physical factors of specimen handling after excision. The explanted material from humans is usually fixed in formalin. Additionally, to expose the surface, the mesh needed to be cleaned to remove ingrown tissue, which was done using chemical methods. These aspects have been points of criticism and it has been questioned whether the methodology could fully exclude artefacts of exposure to chemicals, intra/postoperative handling and residual tissue or biological films. We employed a different methodology by studying cross sections of explanted mesh, without its separation from tissues. This approach allowed us to avoid possible artefacts associated with tissue removal and enabled side-by-side comparison of degraded and non-degraded polypropylene as well as the surrounding tissue components.

MATERIALS AND METHODS

Specimens

After approval of the St. Michael's Hospital Research Ethics Board, 164 consecutive explanted knitted polypropylene mesh specimens received at the pathology department were reviewed. The specimens were from St. Michael's and Shouldice hospital inpatients and outside referrals. Approximately 70% of specimens were potential or active medico-legal cases. More details are provided in Table I.

Specimen processing

The specimens were received as tissue in formalin in 128 cases and paraffin embedded tissue in 36 cases. Tissue received in formalin was processed using Leica TP1020 tissue processor. For all specimens, either received in formalin or paraffin, exposure to formalin was assessed as <72 h in 18 cases, between 72 h and 1 month for 16 specimens, and >1 month for the remaining specimens.

Staining

Tissue was sectioned at 4 μm and all sections were initially stained by haematoxylin and eosin (H&E, Harris haematoxylin). Additionally, 3 random hernia and 7 transvaginal specimens were stained by Masson trichrome and Von Kossa calcium (counterstain neutral red), 10 other random cases stained in combination by Gomori trichrome, Movat, Van Gieson elastin, Ziehl–Neelsen and Grocott's methenamine silver stains.[30,31] Sections of another 5 random explants were stained by immunoperoxidase technique for IgG (DAKO, 1:50 enzyme digestion for 4 min) and 10 explants for myeloperoxidase (DAKO, 1:200 without retrieval) using Ventana Benchmark XT, Gill's haematoxylin.

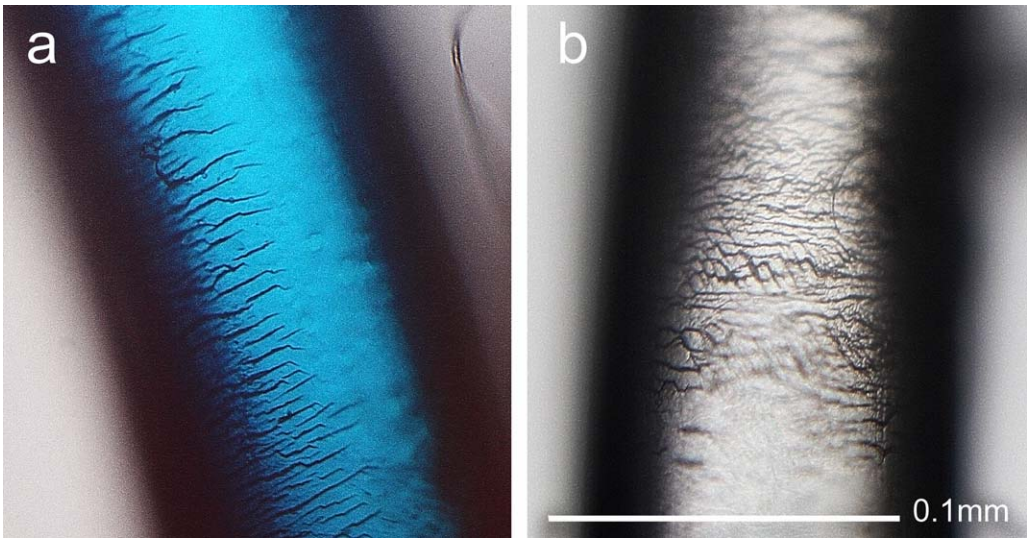

FIGURE 1. Surface of the mesh fibers immediately after explantation from the body, transvaginal sling explanted due to pain 9 years after implantation, light microscope, ×20 objective with image crop. Mesh fibers at the specimen edges had no covering tissue and could be examined as they were in the body, avoiding possible artifacts of tissue removal, drying or contact with formalin. Both blue (a) and clear (b) fibers showed surface cracking. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

New mesh control

Portions of pristine transvaginal sling devices of three different manufacturers were placed in 10% buffered formalin. The mesh was then sampled for light microscopy at 2 weeks and 1, 2, and 4 months in two separate experiments. Tissue processing, embedding, sectioning (charged coated slides) and staining (manual on horizontal tray) were carried out according to the same protocols as for the mesh samples explanted from the patients.

Measurement of degradation layer thickness

Clinical information was reviewed to identify groups of samples from the same manufacturer, the same mesh design, and verified implantation and excision dates. A set of 23 midurethral slings was the largest uniform group that fulfilled these criteria. The sections were examined to find mesh fibers sectioned perpendicularly to their long axis with a cross section close to a near perfect circle to reduce the measurement error of angular orientation. Staining and refractile properties were used to define the edges of degraded material. The thickness of the stained layer was measured with an eyepiece micrometer in at least two fibers with two measurement sites per fiber. The micrometer scale was 1.0 μm at 100× objective with oil immersion and the measurements were rounded to the closest whole number. A median value per specimen was recorded.

Transmission electron microscopy

Subsamples of 1 fresh transvaginal, 6 formalin fixed transvaginal and 1 formalin fixed hernia explant tissue were transferred into glutaraldehyde, then postfixed in osmium tetroxide, dehydrated through a graded series of ethanol, and embedded in a mixture of Epon 812 and Araldite 502. Blue sections were cut and assessed for the presence of mesh fibers in the microblocks. The following 4 samples contained the fibers and were examined using transmission electron microscopy: 1 sample of transvaginal explant fixed fresh in glutaraldehyde, 2 samples of transvaginal explants of another manufacturer transferred from formalin, and 1 sample of hernia explant transferred from formalin. Thin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi 7650 electron microscope.

RESULTS

Light microscopy

In one case the mesh fibers could be assessed immediately after explantation, before tissue drying or fixation in formalin. The transvaginal sling was excised because of chronic pain 9 years after implantation. Mesh fibers at the specimen edges were free of tissue and could be examined in a conventional microscope after a rinse in saline and without additional preparation (Figure 1). There were transverse cracks at the bending points [Figure 1(a)] and patches of haphazard cracks on straighter portions of the fibers [Figure 1(b)].

Microscopic examination of mesh fibers cross-sectioned in the histological slides showed a circumferential outer layer of degraded polypropylene in 162 of 164 examined explants [Figure 2(a)]. Polypropylene degradation was observed across a large range of devices, produced by different manufacturers, explanted from different anatomical locations and due to different clinical complications (Table I). The only two specimens where the degradation layer was not visible were a hernia mesh and a transvaginal sling removed 3 and 10 months, respectively after implantation. As shown further the degradation layer is difficult to detect by light microscopy within the first year after implantation.

The degradation layer was detectable as a rim of purple material in the H&E stained sections while the central core of the fibers remained clear and colorless (except manufacturer's dye). The layer also showed variable staining by other histological stains indicating non-specific trapping of the dyes by

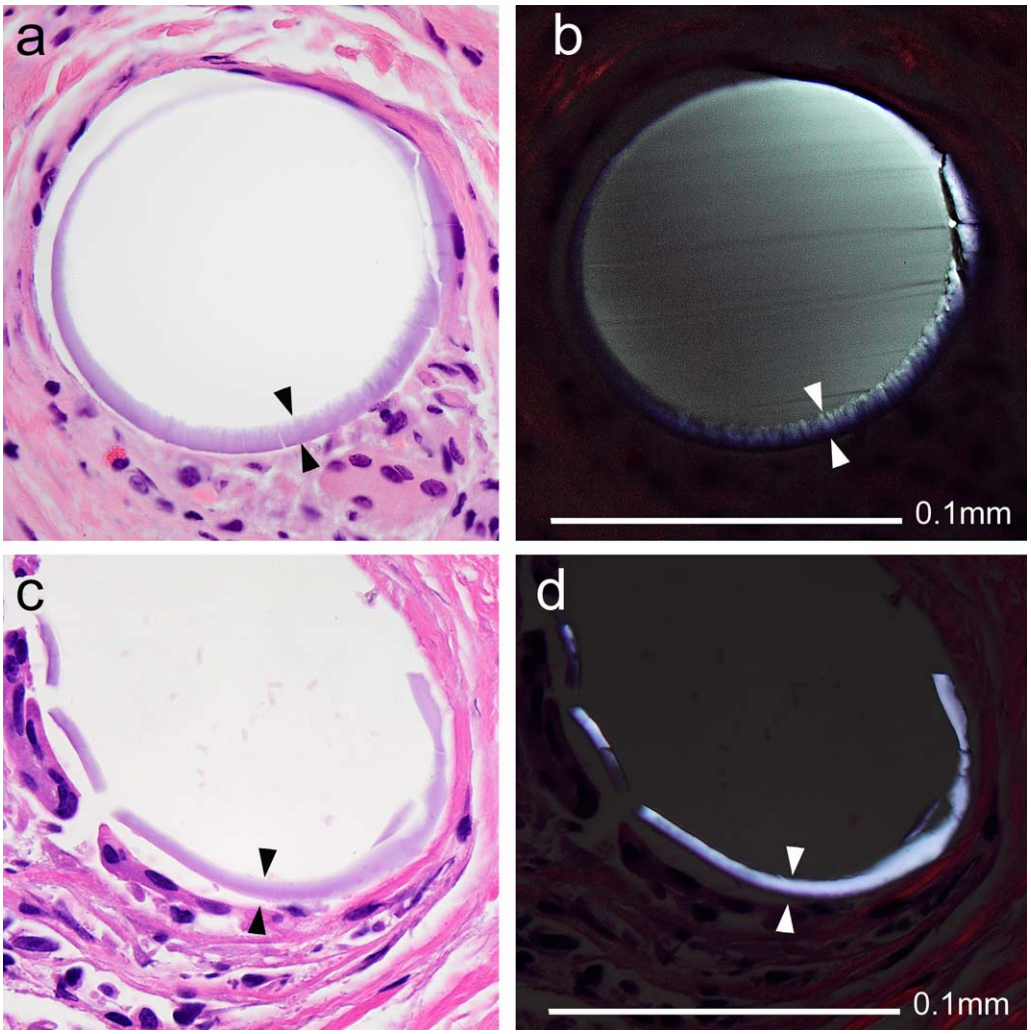

FIGURE 2. Histological sections, H&E stain, ×100 objective with oil immersion. The same cross section of a clear mesh fiber in regular (a) and polarized (b) light. The fiber has a layer of staining degraded polypropylene at the surface (between arrowheads). This layer has refractile properties of polypropylene in polarized light (b). In some areas the non-degraded core of the fibers detaches during histological processing while segments of the degraded layer stay attached to the tissue. Separated degradation layer in regular (c) and polarized (d) light. At sites like this, intensity of light passing through the degraded layer cannot be attributed to light scatter from the core. Note that tissue components including collagen have a much weaker birefringence than polypropylene (b and d). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

van der Waals forces and/or ionic binding. The latter appeared to need a mordant where the alum, but not the iron mordant retained haematoxylin in the layer of degraded polypropylene.

At optical resolution of light microscopy the stainable outer (degradation) layer was homogeneous, without detectable fibrillation. It was of a relatively uniform thickness within individual fibers and of approximately the same thickness between the fibers in the same sample. The layer showed cracks and ability to detach from the non-staining fiber core. It also showed adherence to the tissues. Where the core detached from the glass slide, the outer layer either remained on the fiber or detached from it and stayed adherent to the tissue [Figure 2(c)]. For descriptive purpose, the uniform circumferential nature, fissuring and partial peeling of the layer resembled a tree bark.

We used polarized light microscopy[32] as a routine tool for all 164 specimens to confirm that the stained material was polypropylene. In polarized light, both the central core of the fibers and the outer layer showed similar refractile properties [Figure 2(b)]. The light intensity was uniform within the core while the outer layer had gradual reduction of the refractile ability toward the surface. Birefringence of the outer layer was also observed in the segments of the "bark" separated from the core [Figure 2(c,d)]. At these sites, the light brightness in the "bark" layer was due to its internal properties and could not be attributed to the scatter of light from the core. The adjacent tissue components containing collagen also showed birefringence, however of a much lower intensity. The tissue components also had a different structure and coloration.

Several mesh designs on the market are knitted using a combination of clear and blue fibers. In our pool of specimens, at least 50 explants showed blue fibers. Blue polypropylene fibers incorporated a blue dye as granules introduced

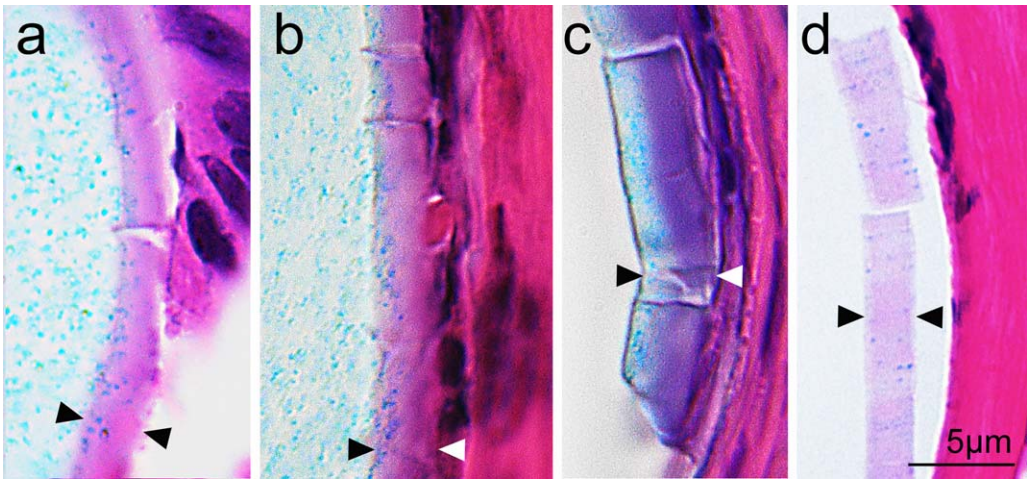

FIGURE 3. Degradation "bark" of the blue fibers manufactured with inclusion of blue dye granules, H&E stain, ×100 objective with oil immersion: (a) and (b) non-degraded core (left half of the images) and the degraded layer (between arrowheads). Note that the blue granules are retained in the layer of degraded polypropylene. Within the degraded "bark," the granules degrade and loose color toward the surface. In (c) and (d) the non-degraded core detached from the slides similarly to Figure 2(c,d). At these sites, presence of the granules in the separated "bark" cannot be attributed to an overlap with the core. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

into the material during its manufacture (Figure 3). The granules were seen in the non-degraded core of the fibers as well as to a variable degree in the outer degradation layer. In the latter, they were detected within the "bark" remaining on the core [Figure 3(a,b)] as well as in the segments of the "bark" separated from the core [Figure 3(c,d)]. The granules were mainly seen in the deeper layers of the "bark" and were not as frequent closer to the surface, which suggests that they also undergo degradation and lose color. The finding was a direct confirmation that the "bark" originated from the same material as the core of the fibers.

The brittleness, staining and birefringence characteristics of the "bark" were similar to those of calcium salts which are commonly deposited in degenerating tissues. Von Kossa stain was used for a sample set of 10 specimens to rule out presence of calcium salts in the outer "bark." No calcium salts were detected in the brittle outer layer of the 10 specimens tested [Figure 4(a)].

The Masson trichrome technique was used to analyze porosity characteristics of the degraded layer in a representative sample of 10 specimens. The trichrome techniques are based on competitive staining by dyes of different molecular

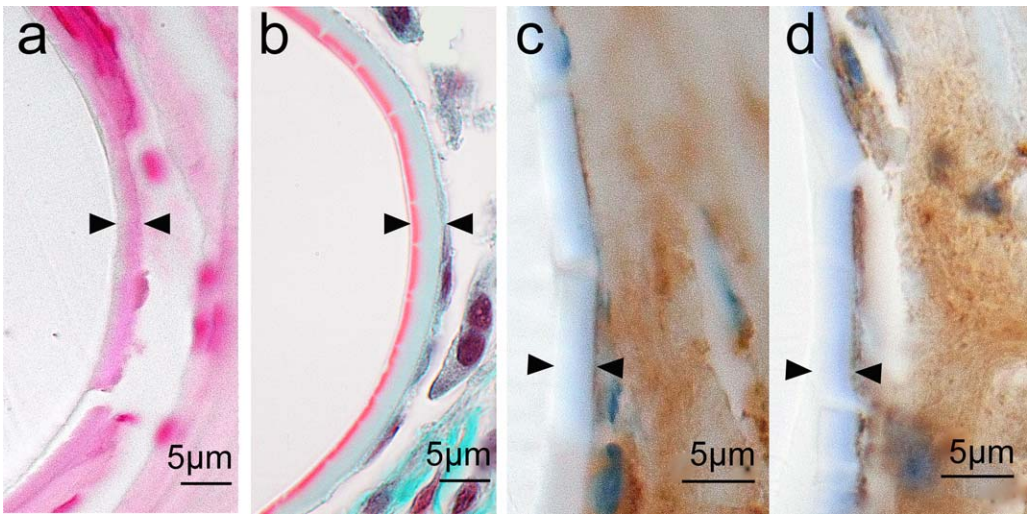

FIGURE 4. Additional stains, all images taken with 100x oil immersion objective and cropped to a different magnification, polypropylene degradation layer is pointed between arrowheads: (a) Von Kossa stain is negative for calcium in the brittle "bark" (would stain calcium black), (b) trichrome stain shows that the deeper parts of the "bark" have smaller staining porosity (red) than those close to the surface (green) which correlates with TEM findings [Figure 6(b)], (c) immunohistochemical stain for immunoglobulin G (IgG, stained brown). IgG is present in almost all human tissues and fluids. It is deposited on the surface of degraded polypropylene but is not mixed within it. (d) Immunostain for the oxidizing enzyme of inflammatory cells myeloperoxidase (stains brown). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

size and penetration ability. A dye (red for Masson) of a smaller molecular size and higher penetration is used in combination with a dye of larger molecular size (green). The stain showed that the deeper parts (close to the core) of the degradation layer had a finer porosity than the more superficial parts [Figure 4(b)]. This was consistent with the findings of transmission electron microscopy demonstrating a network of nanocracks/nanopores expanding toward the surface.

To test whether the outer layer contained proteins, we used immunohistochemical stain for the ubiquitous serum and tissue protein immunoglobulin G (IgG). There was no detectable level of IgG in the bark layer while the immunoglobulin was deposited at its surface [Figure 4(c)]. The finding indicated that IgG came in contact with the fibers; however it was not a component of the "bark" layer as would be expected in a biological film formed by serum proteins.

Myeloperoxidase is an oxidative enzyme expressed by the inflammatory cells together with an array of other oxidative substances. Staining for myeloperoxidase revealed an appearance similar to that of immunoglobulin: the enzyme was detected deposited on the surface of the "bark," but was not observed mixed within it [Figure 4(d)]. This finding further indicated incompatibility of the "bark" material with water-soluble proteins. It also indicated an oxidative environment immediately around the fibers.

Analysis of the effect of surgical cautery on polypropylene

Surgical cautery instruments cause heating of the tissues to a wide range of temperatures. There is usually a narrow zone of high temperature immediately at the tip of the instruments with a sharp drop in temperature in the deeper tissue further away. In excised specimens cautery changes (darkening and nuclear streaming) are seen within 1–2 mm from the cauterized surface. In these areas, mesh fibers showed sites where both the outer degraded polypropylene "bark" phase and the inner non-degraded polypropylene phase melted and mixed to form a single homogeneous phase (Figure 5). The phase-mixed regions did not absorb histological dyes, thereby showing that they lack porosity observed in the degraded polypropylene. The fact that the "bark" was melted during excision surgery indicated that the degradation layer was formed in the body before the excision surgery. The finding also revealed that the fiber core and the outer "bark" are composed of materials with similar chemical compositions that are miscible with each other when heated.

Transmission electron microscopy (TEM)

We used TEM to study the ultrastructural organisation of the degraded layer in cross sections. The mesh fibers showed an outer "bark" similar to that seen by light microscopy [Figure 6(a)]. There were no fibrillary (collagen, amyloid etc.) or other structures of connective tissue matrix within the "bark". Material composition of the outer layer and the core showed similar electron density; however, at high magnification the outer "bark" was noticeably more granular, especially toward the surface [Figure 6(a), insert]. The "bark" material had a lattice of fine branching cracks (nanocracks). This network of nanocracks was sparse closer to the core and expanded toward the surface [Figure 6(b)]. As seen in Figure 6(b) in addition to the nanocracks there were also occasional larger cracks occurring at random. These larger cracks seen by TEM corresponded to the cracks seen by light microscopy either on the surface (Figure 1) or in cross sections (Figure 2).

The "bark" either had a gradual transition into the core [Figure 6(b)] or showed a zone of circumferential fissuring partially separating it from the core [Figure 6(a)]. The mechanisms of "bark" separation from the core and formation of the larger cracks are likely linked since the cracks tended to turn at the interface between the "bark" and the core [Figure 6(b)]. The forces which produced the transverse (radial) cracks also acted as shear forces between the "bark" and the core.

An important finding was the detection of inflammatory cells partially migrated into and trapped within the fissures as shown in Figure 6(c). The shape of widened crevices and the depth of penetration indicated an active *in vivo* cellular migration. This phenomenon was observed at two separate sites.

For better demonstration of the degradation "bark" we used light microscopic and TEM images to generate a 3-dimensional restoration of a mesh fiber (Figure 7).

Thickness of degradation layer versus *in vivo* and *in vitro* intervals

Out of all the specimens, 23 samples of explanted midurethral slings formed the largest group of meshes from the same manufacturer, of identical mesh type and with reliable records of implantation and excision dates. The range of *in vivo* interval (between implantation to excision) in the group was 18–97 months (mean 53). There was a good correlation (Pearson = 0.73) between the thickness of the degradation layer and the duration of *in vivo* exposure indicating that the thickness of degraded material grows while the mesh is in the body [Figure 8(a,b)]. There was a trend for a more rapid initial growth with gradual plateau subsequently.

The "bark" thickness was also analyzed in relation to polypropylene exposure to formalin. Duration of storage of the specimens in formalin ranged from 3 to 32 months (mean 19) for the group. There was no correlation between the thickness of degradation layer and the duration of mesh exposure to formalin (Pearson = −0.06).

Testing of pristine mesh

Samples of three pristine midurethral slings were subjected to formalin fixation followed by tissue processing and H&E staining to reproduce the exposure of explant specimens to the potential factors of postoperative polypropylene degradation *in vitro*. There was no detectable degradation of polypropylene exposed to formalin up to 4 months followed by routine histological processing. This testing showed that exposures to formalin up to 4 months and to the chemical and temperature factors of histological processing do not affect polypropylene to a degree detectable by light microscopy. In comparison, in our pool of mesh explants, 27

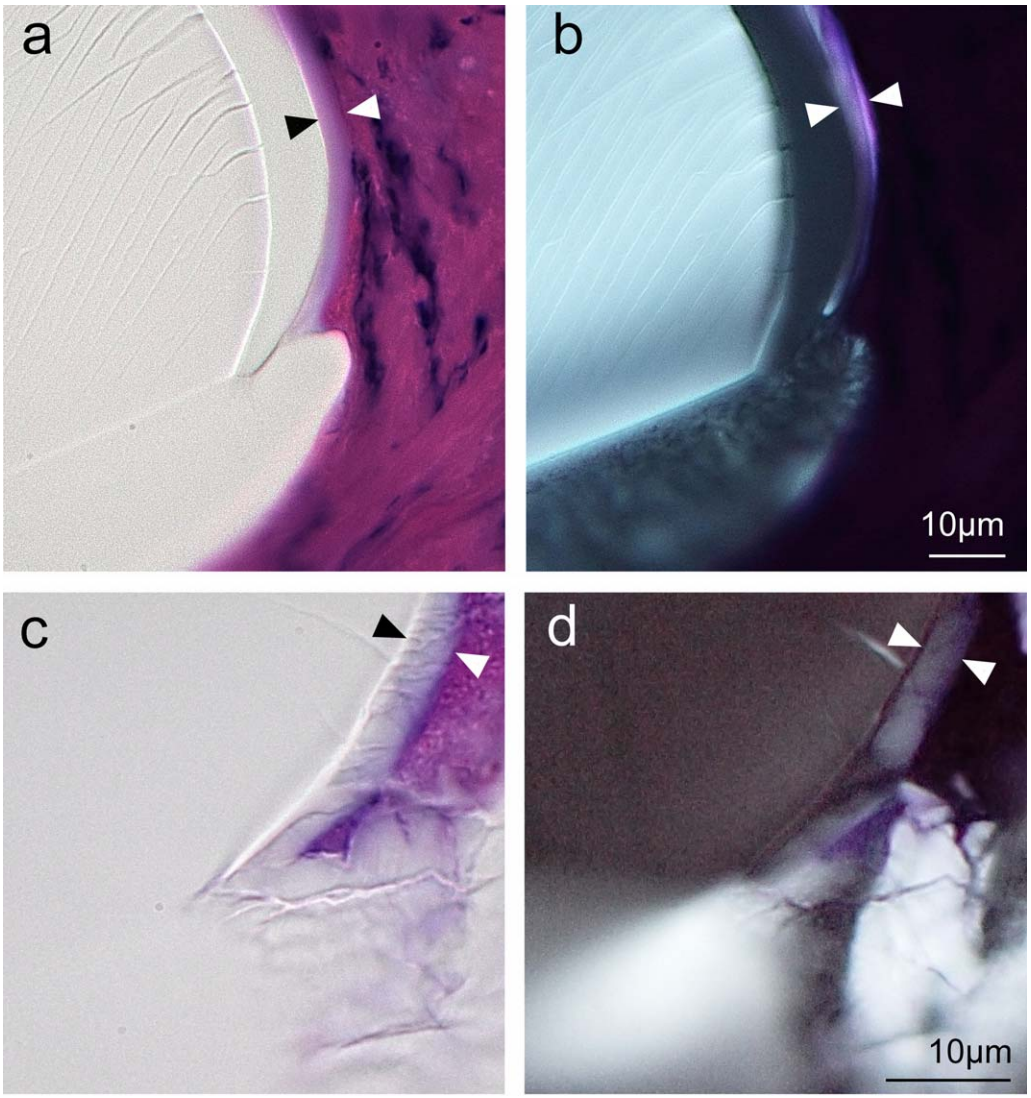

FIGURE 5. Melting of both non-degraded and degraded polypropylene caused by the surgical cautery, H&E, ×100 oil immersion: (a) and (b) the same site of fiber melting in regular and polarized light, (c) and (d) another site showing melding point. While molten the non-degraded core and the degradation "bark" formed a common pool of material. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

specimens had exposure to formalin of <1 month. The findings were in keeping with wide acceptance of polypropylene resistance to formalin for industrial purposes.[33]

DISCUSSION

Previous studies focused on examination of the surface and mechanical properties of explanted fibers and have indicated that polypropylene degrades when exposed to the physiological environment.[16,21–29] These conclusions have been questioned recently,[10,11] while the expanding clinical use and increasing burden of litigation dictated the need to study the mechanisms of complications. Surprisingly, the main source of information, the pathology specimens explanted because of these complications, have not been duly investigated. Using a cross-sectional microscopy approach, we found that the degradation layer is visible in light microscope from a medium magnification power as a rim of stained material around the mesh fibers. In polarized light, the bright curvilinear particles of separated "bark" can be seen from even low magnification. A number of features confirmed that the "bark" is degraded polypropylene (Table II) and that the degradation occurred *in vivo* (Table III). Although the mesh has been in use for several decades, we found no description of these findings in published literature after a search through online and printed sources.

Surface cracking of explanted polypropylene devices has been shown by several reports using scanning electron microscopy.[21–29] In these previous studies, transverse cracks in the direction perpendicular to the axis of the fiber were observed, similar to those observed in the explanted meshes from the present study (Figure 1). We found that these

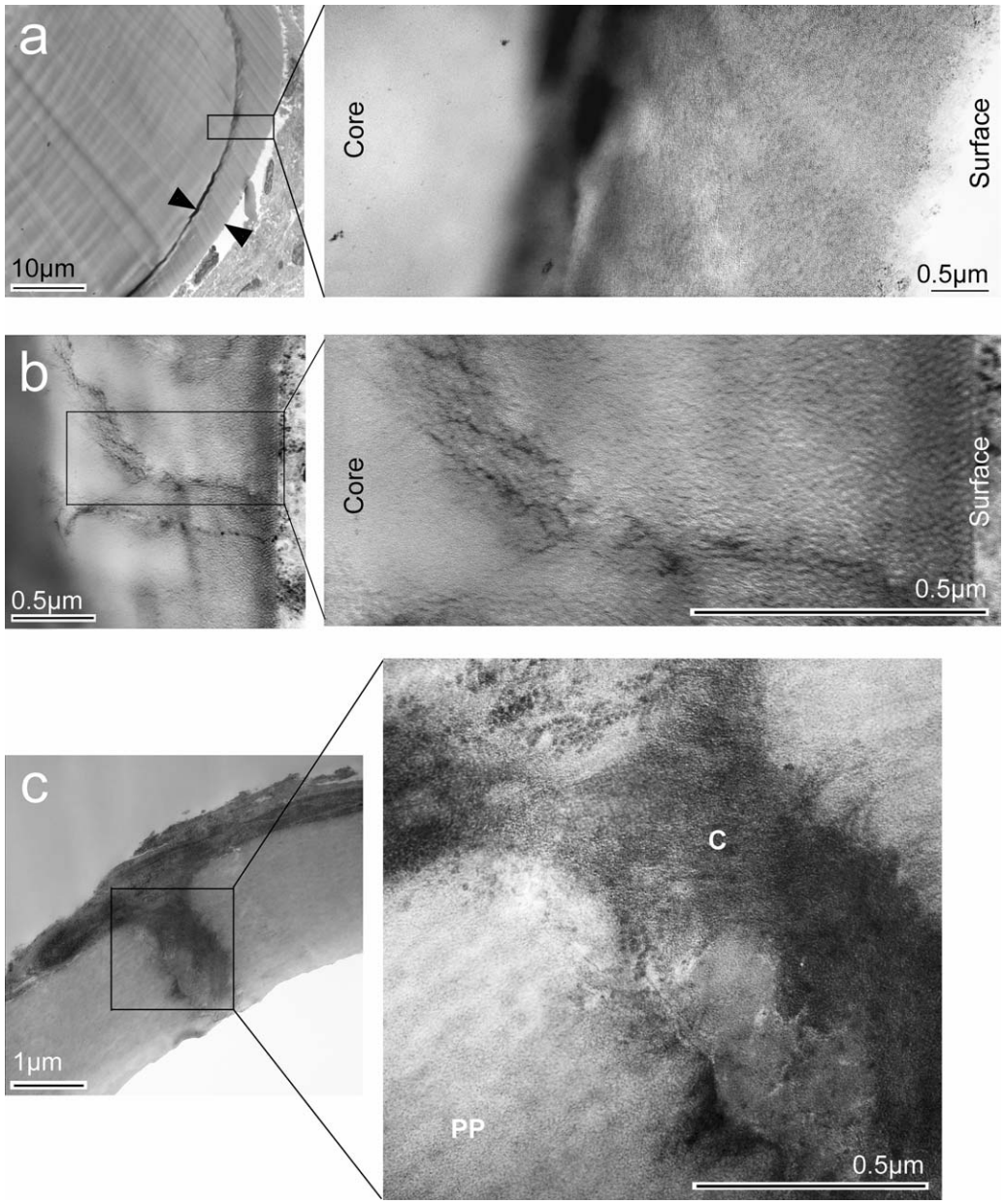

FIGURE 6. Transmission electron microscopy. (a) Low power magnification of a cross-sectioned mesh fiber shows the degradation layer similarly to light microscopy, the insert shows an image composed of three high power fields to reconstruct the transition between the non-degraded core and the degradation "bark." Note the fine nanocracks/nanopores in the degraded material. (b) Outer part of the fiber with gradual transition between the non-degraded core and the degraded polypropylene at the surface. The lattice of nanocracks expands toward the surface (left to right). There are also two larger cracks which start perpendicular to the surface but then turn at the interface between the core and the degraded layer. (c) A part of inflammatory cell (labeled C in insert) is trapped in a fissure within the degraded polypropylene (PP). Higher magnification (insert) shows cellular membrane at the cell-polypropylene interface (left wall of the fissure) and cellular organelles in the cell (chains of rounded structures, likely ribosomes).

cracks can also be observed and studied by light microscopy, which allows for examination of the surface of the fibers immediately after excision, thereby avoiding possible artifacts of drying and chemical treatments.

Previous studies utilizing SEM to characterize surface degradation have been challenged on the basis of the cracks being biological in origin.[11,34] The advantage of cross-sectional analysis by light microscopy and TEM used in the present study is that the degraded "bark," the non-degraded polypropylene core, and the surrounding tissue can be examined in the same section. A variety of histochemical and immunohistochemical stains were utilized to show by light microscopy that the interfacial "bark" layer between the polypropylene core and adjacent tissue was degraded polypropylene. The "bark" layer was further studied using TEM and the examination revealed ultrastructural features

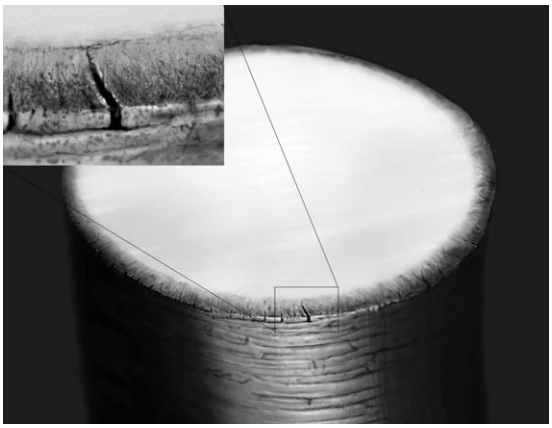

FIGURE 7. A three-dimensional restoration of a cross sectioned mesh fiber.

of degradation. Thus, our findings support the notion that the transverse cracks observed in previous preclinical and clinical studies represent surface degradation of polypropylene.

We observed degradation in all except two explants, one of which was implanted for 3 months and another for 10 months. Thus, the degraded layer does not become reliably visible by light microscopy until about a year after implantation. Similarly, a previous study has reported that polypropylene pelvic meshes explanted from patients <3 months after implantation showed no observable evidence of degradation by SEM.[24] Another study found that unstabilized polypropylene sutures implanted subcutaneously in guinea pigs became chemically induced, which initiates the degradation process after 108 days.[16] For light microscopy, an additional lag time of several months is required for the degraded layer to become detectable in cross-sections and this needs to be considered in future studies.

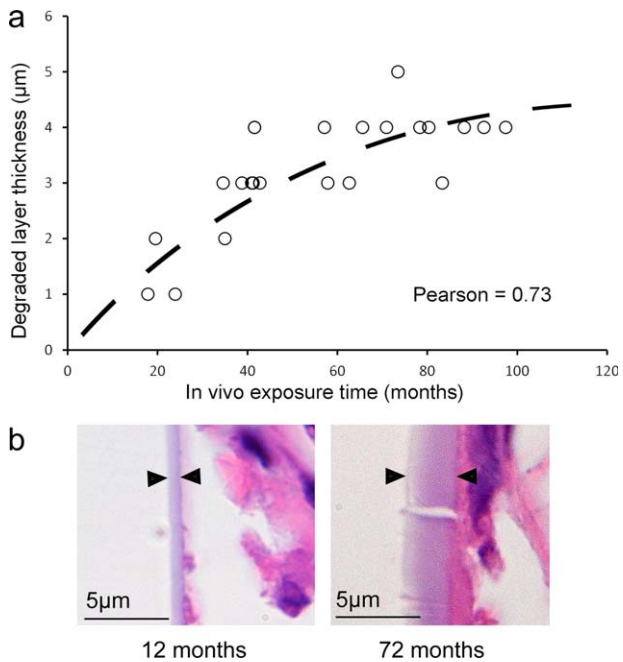

FIGURE 8. Duration of *in vivo* exposure versus thickness of degraded layer in a group of explants of the same manufacturer and the same mesh design. (a) Thickness of the degradation "bark" increased over the years *in vivo* (Pearson correlation = 0.73). Note the trend of plateauing after 5–6 years. There was no correlation of the thickness with the duration of specimen storage in formalin (not shown, Pearson = −0.06). (b) Comparison of the "bark" in meshes explanted after 12 and 72 months in the body, H&E, ×100 objective with oil immersion, images cropped to the same magnification factor. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II. Features Confirming Polypropylene Degradation

Evidence that the outer "bark" is degraded polypropylene
Surface cracking observed in fresh specimen immediately after excision
Birefringence of the "bark" is the same as of the fiber core
Sites of gradual transition between the core and the "bark"
Retention of blue dye granules (introduced during manufacturing) in the "bark"
Sharp demarcation between body proteins and the "bark"
Ability of the "bark" material to meld with non-degraded core at high temperatures
No detectable levels of calcium salts as commonly seen in brittle biological materials
Increasing degree of porosity/degradation toward the surface
No elements of tissue matrix in the "bark" by TEM

Several studies indicated that polypropylene degradation is likely mediated by the foreign body reaction, which is ongoing until the device is removed.[35] Our observations of adherent macrophages on the polypropylene surface are consistent with the previous studies reporting chronic inflammation in explanted polypropylene mesh several years after implantation.[23,24,27,36] We observed strong staining for oxidative enzyme myeloperoxidase produced by the macrophages in the tissue surrounding the mesh fibers. This indicated that, the surface of the polypropylene was exposed to reactive oxygen species (ROS) while oxidation of polypropylene as a result of the foreign body reaction has been suggested as the mechanism of degradation by earlier reports.[24–27,29,36] For many polymers, the rate of oxidation is controlled by the rate of diffusion of molecular oxygen into the polymer.[37–39] We have proposed a mechanism of polypropylene oxidation under simulated *in vivo* conditions

TABLE III. Features Confirming That Degradation Occurs *In Vivo*

Evidence that polypropylene degradation occurs *in vivo*
No detectable degradation of pristine meshes after exposure to formalin and tissue processing
Surface cracking observed in fresh specimen immediately after excision
Gradual increase in "bark" thickness during the years in the body
No correlation between the "bark" thickness and duration of storage in formalin
Entrapment of inflammatory cells and tissue matrix in the "bark" fissures
Melting of the "bark" by surgical cautery (presence before excision surgery)

in which oxidation is initiated by ROS attack on the tertiary hydrogen on the polypropylene surface followed by diffusion of molecular oxygen into the polymer (Talley et al. submitted). In the present study, using a subset of 23 mid-urethral slings from the same manufacturer, we showed that the thickness of the "bark" layer increased with time, in a manner consistent with a reaction-diffusion mechanism.[37]

Clinical significance of polypropylene degradation

It is important to know the effect of degradation of an implanted material on the body and on the long-term performance of the device. When physical and chemical characteristics of a material undergo changes in the body its applications should include planning for safe and complete removal with minimal tissue damage. This exit-strategy is especially important in younger patients, proximity to organs and large vessels, and anatomical sites which are difficult to reach.

The clinical descriptions provided with the specimens indicated that in many cases mesh-related complications develop several years after mesh implantation. The exact mechanisms of these late complications are yet to be understood, however factors accumulating over time need to be considered as primary contributors. As we showed, the degraded layer becomes thicker over time while its cracking indicated brittleness and loss of flexibility. Although the degraded layer is thin in relation to the fiber diameter, its circumferential distribution provides the highest mechanical effect on the mesh fibers. Degradation related stiffening of the mesh is expected to increase over time.

Another clinically important aspect of degradation is the potential for bacterial colonization of the fissures within the degraded material. It is known that irregularities of polymer surface promote bacterial adherence.[40,41]

A described effect of degradation and wear of medical devices is the release of material particles. The debris from prosthetic joints is well known to cause tissue necrosis, inflammation and fibrosis around the joints.[42–44] For polypropylene meshes, we observed occasional particles of degraded polypropylene in the surrounding tissue and macrophages. The load of these visible particles was limited; however we could not test for the presence of smaller particles, not detectable by light microscope, and chemical products of degradation. Outside the body, thermal degradation of polypropylene produces an array of organic molecules such as acids, ketones, ethers, aldehydes, alcohols and smaller hydrocarbons.[45] The conditions of thermal degradation of polypropylene do not match those of degradation in the body; however the results can be used to estimate the range of chemicals that can be potentially produced in the tissue. Additionally, if additives are used to stabilize the polymer or improve its other characteristics, they can also leach into the tissue. Recently, additives leaching from polypropylene labware were shown to affect cultured cells *in vitro*.[46] A systemic effect on humans was detected when intravenous injections of saline from prefilled and stored polypropylene syringes were found to alter smell and taste in pediatric patients.[47] Locally, the molecules released from polypropylene mesh may play role in direct and inflammation-mediated tissue damage with subsequent repair. It has been shown recently that the scar around mesh fibers undergoes a continuous remodelling.[48]

A potential adverse effect of implantable devices is oncogenesis. Oncogenic mechanisms may be related to chemical composition of the device or other factors of device-body interactions. A complicating aspect of studying mutagens is the length of the latency period. For example, radiation induced sarcomas have a median latency period of 10 years with a range 2–50 years.[49]

Because of different developmental pathways, the potential tumorigenic effects need to be analyzed separately for mesenchymal neoplasms, lymphomas, and carcinomas (epithelial tumors). A number of mesenchymal neoplasms have been reported in association with breast and joint prostheses with the latency period of up to 33 (mean 11) years.[50,51] In relation to polypropylene mesh, one case of myofibrobalstic tumor has been reported recently.[52]

With regards to lymphoid cells, specific lymphomas associated with breast implants have been categorized as an entity since primary breast lymphomas are extremely rare without implants.[53,54] The neoplasms develop in association with either saline or silicone filled implants and the exact source of carcinogenicity is not known presently. It may be related to a higher turnover of inflammatory cells surrounding the implants. Median latency period was reported 9 years (range 1–32).[54] There have been no reports of a lymphoma at a site of polypropylene mesh implantation.

Few carcinomas have been reported in association with implantable devices. This risk appears to be related to chronic irritation and inflammation as it is known that the long-standing non-healing wounds are a risk factor for squamous cell carcinoma. Individual cases of squamous cell carcinomas have been reported developing in the chronic wounds of exposed hernia mesh.[55] There appears to be no specific risk for squamous or other type of carcinoma without mesh exposure through the skin.

Based on the analysis of published literature and considering the long term clinical use of polypropylene mesh, the oncogenic risks, if present, are very low. However, continuous introduction of new designs and possible changes of the sources of the raw material pose a potential problem with respect to the variation of chemical composition and new effects on the body.

CONCLUSIONS

The expanded use of polypropylene mesh in clinical practice and the subsequent increase in the number and nature of complications necessitate the study of the mechanisms of those complications. Explants from patients are the primary source of information regarding these mechanisms; however the material has been largely underutilized. We have shown that a focused examination of explanted specimens can reveal features which have been overlooked for decades. Specifically, polypropylene degradation can be detected by readily available conventional light microscopy. A number of

features indicated that polypropylene degrades while in the body. Both physical and chemical aspects of polypropylene degradation need to be studied more extensively for their roles in the development of these complications. The described methods of light microscopy can also be used to study degradation of other materials.

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